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DRUG DISCOVERY

Investigation of chronic anti-inflammatory activity of methanol extract of *Tabebuia hypoleuca* (C. Wright ex Sauvalle) Urb. Stems

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ABSTRACT

Background: Tabebuia spp. has been traditionally used to treatment of different illness. We reported in previous paper the anti-inflammatory activity on acute inflammation models, antinociceptive and antipyretic effect from methanol extract of *Tabebuia hypoleuca* stem. The aim of the present study had been evaluated the chronic anti-inflammatory effect on granuloma induced by cotton- pellet and the anti- arthritis effect from methanol extract of *T. hypoleuca* stem. *Methods:* Cotton pellet –induced granuloma was used as chronic inflammatory model. The granuloma and transudative weights were calculated and recorded the body weight

gain. In addition to that, Complete Freund's adjuvant (CFA)-induced arthritis in rats was used and determinate the paw edema and the body weight. In both experiments were used diclofenac as positive control and methanol extract from *T. hypoleuca* (THME) at 500 mg/Kg. *Results:* Oral administration of THME (500 mg/kg) and diclofenac (5 mg/kg) caused a significant inhibition on the weight of granulomatous tissue and transudative weight compared to control group not affecting the body weight gain. Anti- arthritis effect from THME was demonstrated with a significant inhibition of the paw edema comparable to diclofenac. These evidence, permit to think about development a new anti-inflammatory natural drug with this endemic Cuban plant.

Keywords: *Tabebuia hypoleuca; chronic inflammation; granuloma; arthritis; methanol extract.*

1. INTRODUCTION

Inflammation is a natural protective response of the body to tissue injury caused by chemical, mechanical or thermal stimuli, trauma, microbial agents or autoimmune diseases. Inflammation is therefore a defense mechanism that is vital to health[1, 2, 3, 4]. Acute inflammatory response keeps the integrity of organisms through activation of immune cells[5]. Although, acute inflammation is a protective response of the body, if unresolved, it leads to painful conditions, like rheumatoid arthritis, inflammatory bowel diseases, asthma, allergy, atherosclerosis, immune-inflammatory ailments and even neoplastic transformation [6, 7]. Thus, persistent inflammation is vital factor in the development and progression of chronic diseases[8].

Bignoniaceae are predominantly a neotropical family and are an important component of neotropical forests, with lesser contributions to African, Malagasy, and SE Asian tropical forests. The family includes 82 genera and 827 species[9]. *Tabebuia* spp. (Bignoniaceae) includes approximately 100 species, known as strictly woody, found in tropical rain forest areas throughout Central and South America. Species of the genus *Tabebuia* have been traditionally used to treat syphilis, malaria, cutaneous infections, stomach disorders, cancer, inflammation, pain, bacterial and fungal infections, anxiety, poor memory, irritability, depression, and others[10, 11, 12].

Tabebuia hypoleuca (C. Wright ex Sauvalle) Urb. commonly known as "Roble macho", is an endemic species of Cuba, native to the Sierra Maestra and Guantanamo. Some pharmacological properties have been reported for the methanol extract of *T. hypoleuca* stems (THME) in the last several years, such as, acute anti-inflammatory activity using the carrageenin-induced paw edema models and the croton oil induced auricular edema[13], antinociceptive activity using several models (chemical and thermal) of nociception, and antipyretic activity using brewer's yeast induced pyrexia method[14, 15].

However, there are no studies on anti-inflammatory activity in the chronic phase of *T. hypoleuca*. The present study was carried out to evaluate the chronic anti-inflammatory activity of THME administered orally in rats.

2. MATERIAL AND METHODS

Drugs and chemicals

The extract and all of the drugs were diluted in 0.9% saline solution (NaCl diluted in distilled water). The drugs and chemicals used were: diclofenac (AICA, Havana, Cuba), Complete Freund's adjuvant (Sigma-Aldrich, St. Louis, USA) and methanol (Merck, Germany).

Plant material and extraction

T. hypoleuca stems were collected at the National Botanical Garden (JBN), Havana Province, Cuba, in June 2017. The identification of the plant was confirmed by Dr. Eldis R. Becquer and a sample was deposited in the herbarium of the experimental station with the number HFC-88204. Stem were separated, dried at room temperature by one week and 48 h at 37°C. Dried stems were milled to 40 mesh. Solid-liquid extraction in Soxhlet with methanol (Merck®) was used for the extraction of *T. hypoleuca* stems. The methanol extract was filtered and concentrated using rotary evaporation.

Animals

Male Sprague-Dawley rats with 180-200 g body weight were supplied by the National Center for Laboratory Animal Production (CENPALAB, Santiago de Las Vegas, Havana, Cuba). The animals were kept under standard conditions of 23 ± 2 ° C, 40–60% relative humidity, and a 12/12 h light-dark cycle, and they were given food and water ad libitum for 7 days. All experimental procedures were performed in accordance with the International Guidelines for the Care and Use of Laboratory Animals and approved by the Animal Ethical Committee of the National Center for Animal and Plant Health (CENSA, Havana, Cuba).

Chronic anti-inflammatory activity

Cotton pellet-induced granuloma

The cotton pellet induced granuloma method was performed as described by Swingle and Shideman (1972). The granulomatous lesions were induced by surgically implanting two cotton pellets subcutaneously in the dorsal region of the rats, one near each axilla. Male rats were divided into three groups of six rats each. The animals were treated orally with diclofenac (5 mg/kg), distilled water (10 ml/kg), and THME (500 mg/kg). Sixty minutes after administration, each rat was anaesthetized with ether and autoclaved sterile pellets of cotton, weighing 20±1 mg each, were aseptically implanted in the interscapular distance under the skin on the previously shaved back of the rats. The animals were treated once daily for 7 days and on the eighth day, all the rats were sacrificed and cotton pellets were carefully removed and made free from extraneous tissues, it was dried at 60 °C for 24 h. The pellets were weighed in both moist and dry condition. The granuloma and transudative weights were calculated. The body weight gain was also recorded. The percentage inhibition of granuloma formation was calculated by the following equation:

$$\text{Percent Inhibition} = \frac{\text{Weight of pellet (control)} - \text{Weight of pellet (test)}}{\text{Weight of pellet (control)}} \times 100$$

Freund's complete adjuvant-induced arthritis

Experimental arthritis was performed according to the method proposed by Latha et al., (1998)/Newbould (1963)[16, 17]. Arthritis was induced in rats by a single injection of 0.1 mL of Freund's complete adjuvant (FCA), containing 1 mg/mL of heat-killed and dried *Mycobacterium tuberculosis* in paraffin oil and mannide monooleate (Sigma-Aldrich, St. Louis, USA) in to subplantar region of left hind paw on day 1. The animals were anaesthetized with ether, as the viscous nature of the adjuvant exerts difficulty while injecting. Male rats were divided in four groups of six animals by group. From the next day, one group was considered as normal control group without arthritis, the second group were treated with distilled water (10 ml/kg), another one the rats were treated orally with diclofenac (5 mg/kg), and the last group treatment with THME (500 mg/kg, p.o), and were administered daily until 21 days after the treatment of CFA. Paw and ankle diameter of all groups of animals were measured with the help of digital calipers on day 1th, 7th, 14th, and 21st. The percentage inhibition of was calculated by the following formula:

$$\text{Percent Inhibition} = \frac{(Ct - Co)\ \text{Control} - (Ct - Co)\ \text{Treated}}{(Ct - Co)\ \text{Control}} \times 100$$

Statistical analysis

Statistical analysis was performed using the statistical software package SPSS (version 21.0). Data were expressed as mean ± SEM. One-way ANOVA followed by the Dunnett post hoc test was used to determine the significant differences between the control and treatment groups. $p < 0.05$ was considered statistically significant.

3. RESULTS

Chronic anti-inflammatory activity

Cotton pellet-induced granuloma

Oral administration of THME (500 mg/kg) and diclofenac (5 mg/kg) caused a significant inhibition ($p < 0.01$ and $p < 0.001$, respectively) of the weight of granulomatous tissue compared to the control group (Fig. 1). The percentage inhibition of granuloma tissue formation for THME was 37%, and for diclofenac was 51%.

In this model, THME at the dose of 500 mg/kg and diclofenac at a dose of 5 mg/kg significantly reduced ($p < 0.01$ and $p < 0.001$, respectively) the transudative weight compared to the control group, with 21% (THME) and 34% (diclofenac) inhibition (Fig. 2). In addition, a normal body weight gain was observed in all groups (Fig. 3).

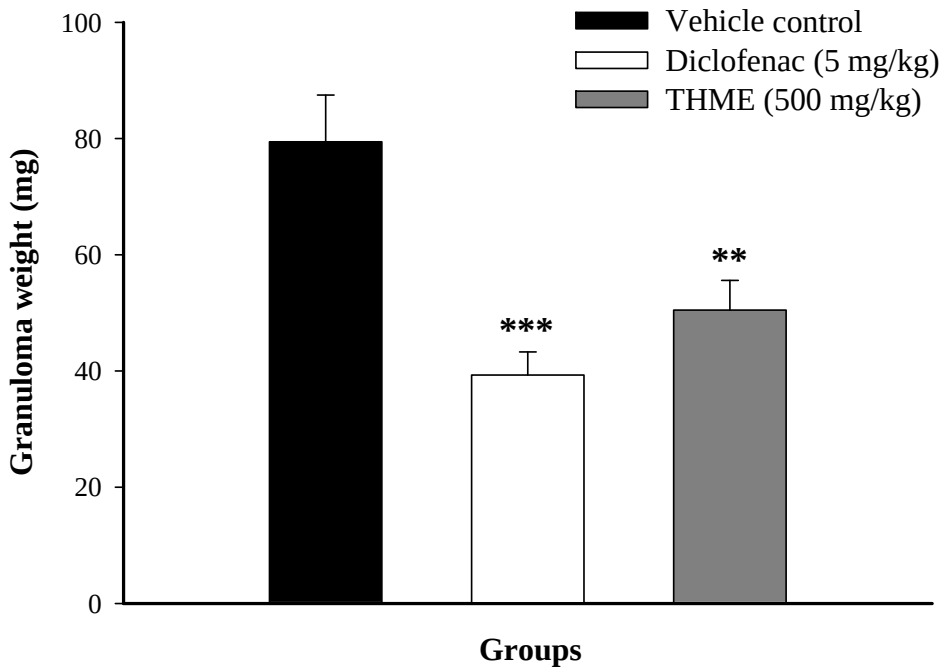


Figure 1 Effect of the oral administration of THME (500 mg/kg) and diclofenac (5 mg/kg) on weight of granuloma formed in the cotton pellet-induced granuloma model in rats. The results are presented as mean ± SEM (n=6). ** $p < 0.01$ and *** $p < 0.001$ vs. control group (one-way ANOVA followed by Dunnett test).

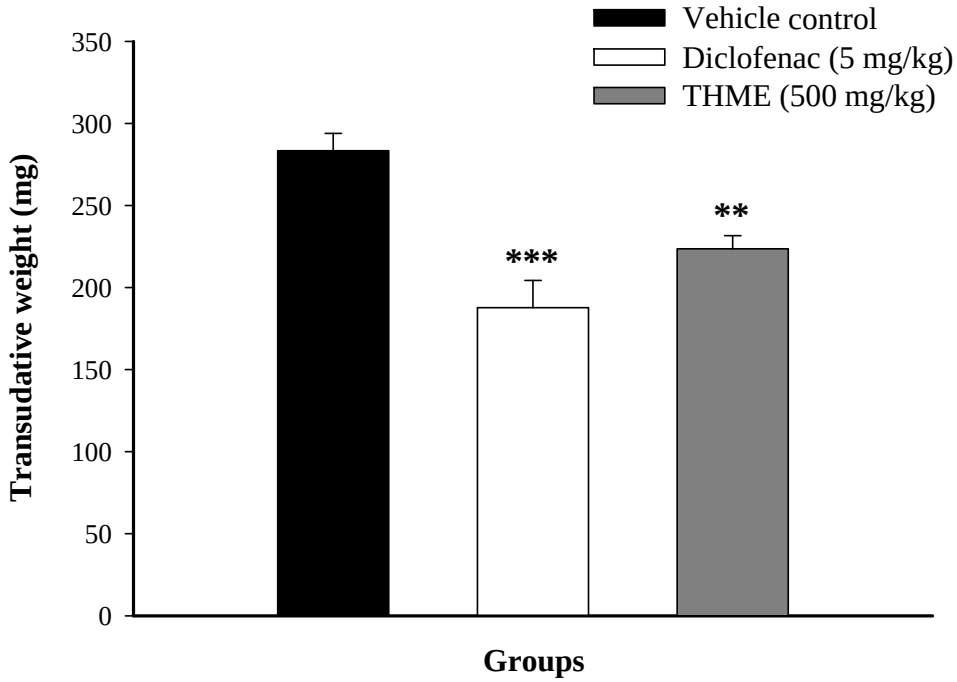
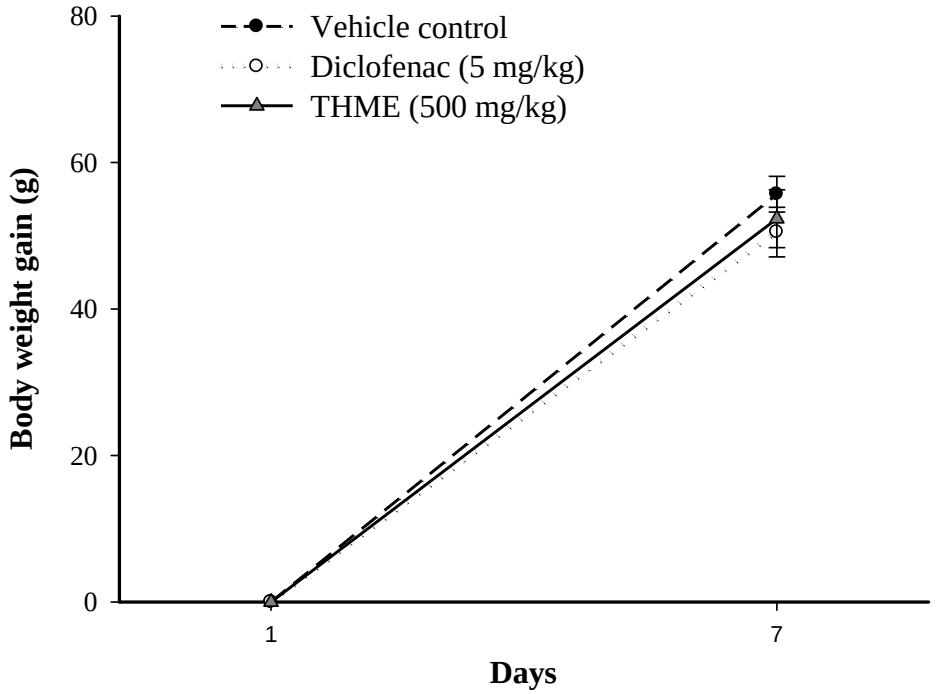


Figure 2 Effect of the oral administration of THME (500 mg/kg) and diclofenac (5 mg/kg) on transudative weight in the cotton pellet-induced granuloma model in rats. The results are presented as mean ± SEM (n=6). ** $p < 0.01$ and *** $p < 0.001$ vs. control group (one-way ANOVA followed by Dunnett test).



Figore 3 Effect of the oral administration of THME (500 mg/kg) and diclofenac (5 mg/kg) on body weight gain in the cotton pellet-induced granuloma model in rats. The results are presented as mean ± SEM (n=6).

Freund's complete adjuvant-induced arthritis

In this test, rats injected with FCA showed an increase in paw diameter when compared to the normal rats. Oral administration of THME at the dose of 500 mg/kg showed significant (p < 0.001) reduction (36.36% inhibition) in the paw diameter of rats compared to the control group, 7 days after the induction of edema with Freunds' complete adjuvant. Also, the paw diameter of rats of the positive control group treated with diclofenac sodium (5 mg/kg, p.o.) was significantly (p < 0.001) lower (36.36% inhibition) compared to the control group (fig. 4).

As illustrated in Fig. 5, there was a progressive increase of body weight gain throughout the experiment in all groups.

4. DISCUSSION

In a previous study, the stems of THME at doses level of 150, 300 and of 500 mg/kg was evaluated in carrageenan paw oedema and croton oil induced auricular edema models, where the stem extract was found to show significant anti-inflammatory activity by oral administration. In the study, was demonstrated that THME at 50 mg/kg intraperitoneally had a superior anti-inflammatory activity respect to indomethacin (73.65 and 55.67 % of edema inhibition, respectively)[13]. Anti-nociceptive and antipyretic properties were demonstrated by THME at 500 mg/Kg [14, 15].

For these previous results, it was selected the single doses of the 500 mg/Kg by the chronic anti-inflammatory studies from methanol extract of *Tabebuia hypoleuca* stem.

When the acute inflammatory response fails to eliminate the causative agent or to restore the normal physiology of the injured tissue, a chronic state of inflammation occurs, which is characterized by infiltration of lymphocytes and macrophages and can lead to a proliferative phase accompanied with tissue changes (formation of new capillaries and proliferation of fibroblasts). A morphological type of this stage is granulomatous inflammation, characterized by an organized collection of macrophages. The cotton pellet-induced granuloma is a representative model for studying drugs against this inflammation phase: the granuloma formed by day 7 is characterized by the formation of a vascularized fibrous capsule containing fibroblasts and infiltrating mononuclear cells[18, 19].

In chronic inflammatory response, accumulation of fibroblasts and the persistence of inflammatory cells lead to the release of pro-inflammatory mediators, free radicals, and lysosomal enzymes such as ALP which result in subsequent tissue injury[20]. Macrophages accumulate in layers surrounding the problematical material and sometimes form giant cells. The structure is formed, with layers of macrophage surrounding a central core, called a granuloma[21].

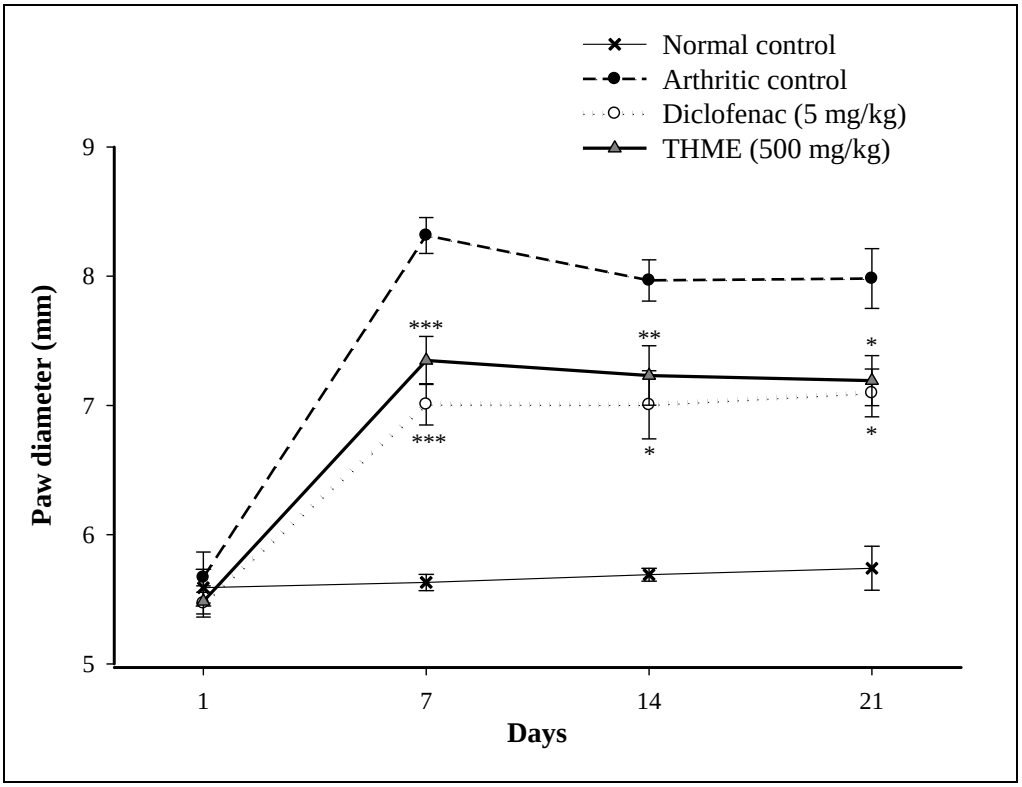


Figure 4 Effect of the oral administration of THME (500 mg/kg) and diclofenac (5 mg/kg) in the writhing test. The results are presented as mean ± SEM of the number of writhes (n=8). ***p < 0.001 vs. control group (one-way ANOVA followed by Dunnett test).

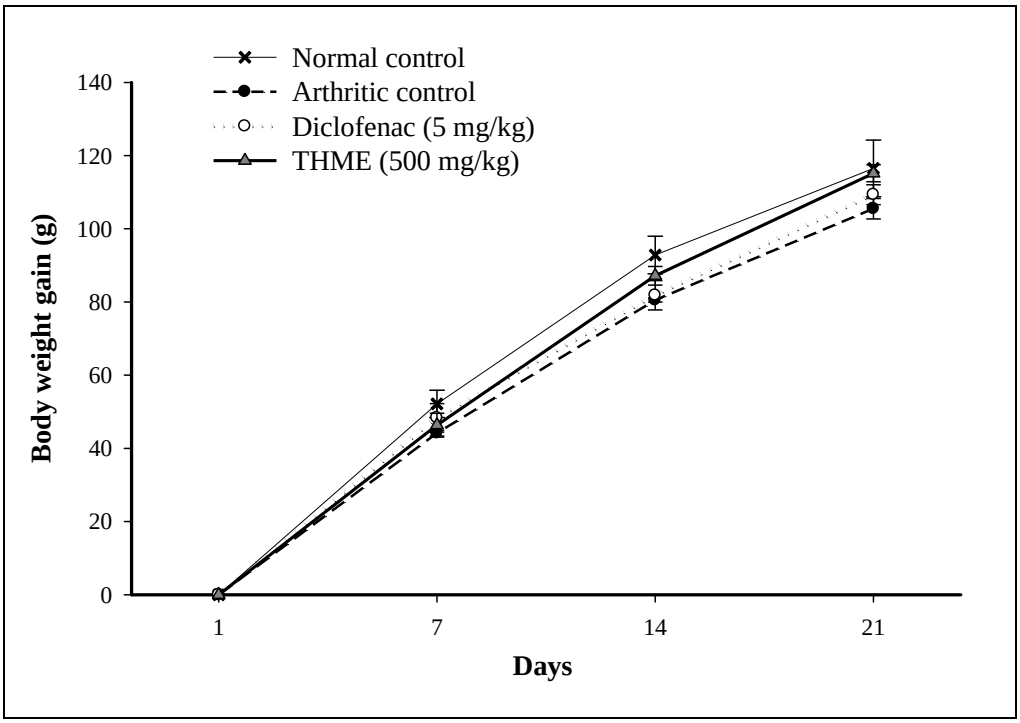


Figure 5 Effect of the oral administration of THME (500 mg/kg) and diclofenac (5 mg/kg) in the arthritis test. The results are presented as mean ± SEM of the number of writhes (n=8). ***p < 0.001 vs. control group (one-way ANOVA followed by Dunnett test).

The cotton pellet-induced granuloma is widely used to assess the transudative and proliferative components of chronic inflammation. The weight of the wet cotton pellets correlates with transude material and the weight of dry pellet correlates with the amount of granulomatous tissue. Three phases of the inflammatory response to a subcutaneously implanted cotton pellet in rats have been described. A transudative phase that occurs during first 3 h, an exudative phase occurring between 3-72 h after implanting the pellet and proliferative phase measured as the increase in dry weight of granuloma that occurs between 3 and 6 d after implantation. The suppression of proliferative phase of sub-acute inflammation could result in a decrease in weight of granuloma formation. It is well-known fact that diclofenac sodium act by inhibiting the prostaglandins synthesis at the late phases of inflammation. This effect may be due to the cellular migration to injured sites and accumulation of collagen, an important mucopolysaccharide[22]. Decreasing granuloma tissue, prevention of occurring of the collagen fiber and suppression of mucopolysaccharides are indicators of the antiproliferative effect of NSAIDs[23].

THME showed anti-inflammatory effect on the chronic inflammatory process by inhibiting the cotton-induced granuloma formation. THME caused significant inhibition percentages 37% and markedly reduced the dry weight and the wet weight of the cotton pellet at a dose of 500 mg/kg when compared to control. The standard drug diclofenac produces maximum activity by inhibiting the wet weight and dry weight of the cotton pellet, 51 % and 34 % respectively. Any toxic effects were shown by THME and diclofenac respect to negative control group with a well weigh gain. Thus, our finding suggests that THME could be used as a potential agent in treatment of chronic inflammatory disorders.

Rheumatoid arthritis (RA) is a chronic inflammatory autoimmune disease with the main clinical manifestation of systemic complications, including synovial inflammation, joint lesions, and bone damage. It is a clinical syndrome that includes several disease subsets and usually involves several inflammatory cascades, finally leading to persistent synovial inflammation and associated damage to articular cartilage and underlying bone. Humans produce a large number of adverse substances in response to a harmful stimulus, which may further induce RA[24, 25]. Currently, 0.5%–1% of people around the world suffers from arthritis[26].

Freund's complete adjuvant induced arthritis model is extensively used to study the pathogenesis of rheumatoid arthritis for testing therapeutics and this model is characterized by a very rapid erosive disease[27]. Freund's Complete Adjuvant is inactivated and dried mycobacteria which are mainly responsible for stimulation of cell-mediated immunity which ultimately increased the production of certain immunoglobulins. FCA induced arthritis is a primary and secondary chronic arthritis[28]. Primary is inflammatory phase where generation of prostaglandin occurs and secondary immunological state in which autoantibodies is generated. Release of various inflammatory mediators including cytokines (IL-1B and TNF-alpha), MCSF, interferon's and Platelet derived growth factor (PDGF) are responsible for the initiation of pain along with swelling of the limbs and joints, bone deformations and disability of joint function. Significant increase in the paw thickness after sub plantar administration of FCA is reflecting the status of arthritis[29]. Significant increase in the paw thickness after subplantar administration of FCA is reflecting the status of arthritis. Treatment with THME significantly decreased the thickness of paw via inhibition of release of inflammatory mediators, indicating its anti-inflammatory potential in FCA induced arthritis.

Health status and disease recovery is indirectly concerned with body weight. Body weight, food intake and metabolism are affected by immunity and inflammation and they are regulated by a cytokine-like hormone known as Leptin. In FCA induced arthritis, within 24 hrs of the administration of FCA the plasma leptin levels were rapidly increased which led to anorexia and body weight loss[30]. With the increased severity of arthritis the body weight also reduced significantly. Our finding of the present investigation demonstrated that the treatment with THME significantly attenuated this decreased body weight in FCA induced arthritis.

Previously, we reported the presence of hopenone b (moretenone) as a major compound present in the methanol extract from the *Tabebuia hypoleuca* stems and taraxerone and lupenone as minor compounds[31]. Moretenone have been reported as anti-inflammatory by different authors[32, 33, 34]. Pérez et al., 2018[35] demonstrated the anti-inflammatory activity on chronic inflammation models.

These results show that *Tabebuia hypoleuca* plant would be an effective in long-term anti-arthritic agent to overcome serious side effects synthetic agents. However, further studies are needed to carry out the identification of the other compounds present in the methanol extract and to define the inflammation mediators that this plant and its compounds offer that anti-inflammatory activity.

5. CONCLUSION

The present study demonstrated that methanol extract from *Tabebuia hypoleuca* stem has significant anti-inflammatory action in two chronic experimental models, comparable to diclofenac. This species and their constituents could represent in the future a new

therapeutic option for the treatment of inflammatory diseases. For this reason, we recommend further studies to confirm its anti-inflammatory activity through inflammatory mediator's determination and isolating other bioactive ingredients that will be responsible for the anti-inflammatory activity.

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Conflict of Interest:

The authors declare that there are no conflicts of interests.

Peer-review:

External peer-review was done through double-blind method.

Data and materials availability:

All data associated with this study are present in the paper.

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